PHARMACOKINETICS AND DISPOSITION OF ^{125}I-THYROXINE, ^{125}I-NaI, AND ^{125}I-CYT. C POLYMERS IN RATS AFTER I.V. INJECTION.

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The First priority for studying on pharmacokinetics and disposition of drugs is to elucidate clearly in vivo behavior of ^{125}I-NaI and some suitable polymers such as cyt. c. having no definitive and bioactive properties among monomer or polymers, respectively. Our presentation concerns different pharmacokinetics and dispositions of ^{125}I-NaI in rats with/without premedication of KI water. ^{125}I-cyt. c polymers were used for estimating protein sizes possible to pass through biliary and nephritic barriers with time after i.v. injection. Results show particularly different tissue distribution of ^{125}I-thyroxine from ^{125}I-NaI with no premedication of KI through a relatively early period (0-2h) and much retention of ^{125}I in tissue and organs 24 hr. after i.v. injection in the case of no premedication of KI. In bile, monomer, dimer, trimer, tetramer followed by pentamer of ^{125}I-cyt. c were detected with time after i.v. injection, but a relatively small polymers such as monomer and dimer were detected in urine.
Discussions will be presented in regard to a few reasons why big sizes of some bioactive protein polymers have been trapped in renal tubules.

HUMAN LIVER MICROSOMAL GLUTATHIONE TRANSFERASE
Studies on the substrate specificity, activation by trypsin, isoelectric point, amino acid composition and primary sequence.

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Human liver microsomal glutathione transferase was purified by a modification of the method used to purify the rat (Morgenstern et. al.) and human enzyme (McLellan et. al.). The enzyme displayed the following glutathione peroxidase activities towards dilinoleoylphosphatidylcholine hydroperoxide (0.03, 0.17 µmol/min mg), linoleic acid hydroperoxide (0.09, 0.15 µmol/min mg), cumene hydroperoxide (0.04, 3 µmol/min mg) and methyl linoleate ozonide (0.02, 1.2 µmol/min mg) with the unactivated and N-ethylmaleimide activated enzyme respectively. The activity towards 1-chloro-2,4-dinitrobenzene was 1.9 and 24 µmol/min mg for untreated and activated enzyme. These activities are comparable to or lower than the activities of the corresponding rat enzyme. Human liver microsomal glutathione transferase displays an isoelectric point of 9.4 and a subunit molecular weight of approximately 17.3 kD (deduced from sodium dodecyl sulphate-polyacrylamide gel electrophoresis). The amino acid analysis results and sequence analysis of the N-terminal 17 amino acids agree with the amino acid sequence deduced from the human cDNA sequence. Gel electrophoretic analysis revealed that proteolytic activation of human microsomal glutathione transferase by trypsin is accompanied by cleavage in the Lys-41 region. Human liver microsomal glutathione transferase is similar to the rat enzyme in its activity profile and ability to become activated by N-ethylmaleimide and trypsin. Functional characteristics such as regulation and possible ability to prevent lipid peroxidation can therefore be expected to be the same. Furthermore, expression of the primary sequence deduced from the human cDNA is now supported at the protein level.

Morgenstern, R., Guthenberg, C. and DePierre, J. W. (1982) Eur. J. Biochem. **128**, 243-248.
McLellan, L. I., Wolf, C. R. and Hayes, J. D. (1989) Biochem. J. **258**, 87-93.

RADIOLUMINOGRAPHY FOR QUANTITATIVE AUTORADIOGRAPHY OF ^{3}H

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X ray film has been practically only one photo sensitive material for 150 years since use of X rays in scientific fields. Now it is time to exchange into "Radioluminography" from X-ray film. A new combination of a new photosensitive material, so called Imaging plate (IP) and a new computed Imaging Analyzer System is presented, hereby, as a new term of "Radioluminography". Displayed images by Radioluminograph can give completely quantitative data for macro-autoradiography of tritium. Our presentation is in regard to principles why Radioluminography is able to give the quantitative data for ^{3}H, with some examples including not only whole body auto-radiographs but also TLC- and PAGE (poly acryl amide gel electrophoresis) -autoradiographs. The most characteristic specificities of "Radio-luminography" are caused by its most accurate quantitativeness, its highest sensitivity, and its most skillful computed functions than ever before. By use of a trial IP for 3H, a linear relationship between ^{3}H-radioactivity and PSL(photo stimulated luminescence) intensity is shown clearly under the condition of radioactive concentration of 0.5nCi to 1000 nCi/mg for 2 hr. exposure. The highest sensitivity is shown by 0.1 nCi/mg for 1 hr. exposure and that is a property which has not been expressed ever before.

(R)-IBUPROFEN-S-COENZYME A SYNTHETASE ACTIVITY IN HEPATIC SUBCELLULAR FRACTIONS FROM RAT, MOUSE, HAMSTER AND GUINEA-PIG.

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The metabolic chiral inversion of (R)-ibuprofen is known to involve stereospecific formation of an acyl-CoA intermediate and its subsequent racemisation and hydrolysis. The purpose of this study was to quantify (R)-ibuprofen-S-CoA synthetase activity in subcellular fractions from rat, mouse, hamster and guinea-pig so that differences in both the amount of activity and in its subcellular localisation could be identified. Mitochondrial, microsomal and cytosolic fractions were prepared and incubated with 2 mM (RS)-ibuprofen, 1 mM CoASH, 10 mM ATP in 50 mM HEPES/ 5 mM dithiothreitol/ 15 mM $MgCl_2$ (pH 7.4) for 10 min at 37°C. (R)-ibuprofen disappearance was measured by enantiospecific high performance liquid chromatography (HPLC). No activity was detectable in the mitochondrial or cytosolic fractions from any species tested (limit of detection 25 nmol/h/mg). The activities in the microsomal fractions under these conditions (in nmol/h/mg, mean $\pm$ SEM.) from the various species were as follows; rat (264 $\pm$ 33.3, n=5), guinea-pig (206 $\pm$ 4.3, n=4), hamster 56 $\pm$ 6.0, n=3), mouse (not detectable). Both CoA and ATP were required for activity. The acyl CoA product was isolated by reverse-phase HPLC and its identity confirmed by mass spectrometry. There was no change in the concentration of (S)-ibuprofen in any of the incubations, showing that inversion/hydrolysis of the acyl-CoA intermediate required factors absent under these conditions. These preliminary results demonstrate clearly the existence of species differences in hepatic (R)-ibuprofen-S-CoA synthetase activity, the nature of which warrant further investigation. (M.T. Moxham was supported by a SERC CASE studentship).

THE EFFECTS OF BUTYLATION ON THE FATE OF D-SERINE IN MALE AND FEMALE RATS

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The distribution, metabolism and excretion of [^{14}C]-D-serine was compared with that of the substituted analogue [^{14}C]-tert-butyl-D-serine (TBDS) in male and female rats up to 5 days following intravenous administration (0.5 umoles/Kg). The majority of D-serine (55% of dose), was metabolised to $^{14}CO_2$ (within 48 hrs), 26% of the radioactivity was eliminated in urine and 2.5% in faeces. By contrast, following [^{14}C]-TBDS administration 90% of the dose was rapidly (<24 hr) excreted in urine and only 2% was eliminated in faeces. Urinary excretion was more rapid in females than in male rats over the first 6 hrs (41% as compared to 15% of dose respectively). The major urinary metabolite was shown to be N-acetyl-[^{14}C]-tert-butyl-D-serine. This metabolite accounted for 40% of dose in males as compared to 15% in female rats.

Whole body antigens showed a characteristic accumulation of radioactivity in kidney and pancreas for both compounds. Quantitative analysis of these tissues following [^{14}C]-TBDS administration showed unchanged parent and the N-acetyl derivative.

RELATIONSHIP BETWEEN STRUCTURE AND THE INCORPORATION OF BIPHENYLCARBOXYLIC ACID ANALOGUES AS GLYCERIDES.

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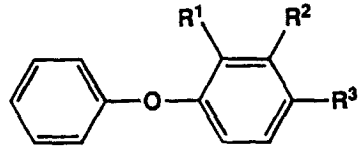
Recently we have been studying the relationship between the structure of biphenylcarboxylic acid derivatives and the rate at which they are incorporated as glycerides using rat liver slices. In typical studies, derivatives of the type (1), substituted at C4, were incorporated as glyceride to a much higher extent than the C2 substituted compound (2).



(1):R^1,R^2=H;R^3=COOH
(2):R^1=COOH;R^2,R^3=H

Similar results were observed within several series of compounds of this type. Proposals to explain this differential incorporation, in relation to the structure of the substrate, will be discussed.

IV PHARMACOKINETICS OF ISOTRETINOIN IN THE RAT

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Isotretinoin (13-*cis*-retinoic acid) is a retinoid used clinically for the oral therapy of severe acne and is also recommended for severe Gram-negative folliculitis and roseacea not responding to traditional therapy. The aim of this study was to characterize the pharmacokinetics of 13-*cis*-retinoic acid in the anaesthetized rat.
Two groups of 5 male Wistar rats (200-250g) were anaesthetized using sodium pentobarbitone. Isotretinoin was administered at two doses *via* the left jugular vein in an emulsion of soya oil. Blood samples were collected over a period of 210 minutes from the right carotid artery. Isotretinoin was extracted from plasma by a direct precipitation method using acetonitrile. After vortex mixing the samples were centrifuged and the supernatant layer was directly injected onto a reversed phase hplc system.

Dose (mg/kg)	0.264	0.792
AUC (μg/ml.min)	55±31	317±34
Elimination half-life (min)	48±5	61±7
Clearance (ml/min/kg)	6.0±3.4	2.5±0.2
Volume of distribution (ml)	421±227	222±19

A non-proportional increase in AUC was observed with increasing dose, resulting from a decrease in volume of distribution and clearance. The elimination half-life also varied with increasing dose. A large degree of inter-subject variation was seen in the pharmacokinetic data.
The financial support of the SERC and Roche Products Ltd, UK is gratefully acknowledged.

THE ROLE OF CYTOCHROME P450 IIIA IN THE METABOLISM OF FELODIPINE AND ALDRIN BY HUMAN LIVER MICROSOMES.

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Several therapeutically important agents have been shown to be metabolised by the cytochrome P450 IIIA subfamily. These include cyclosporin A and the 1,4-dihydropyridine calcium channel antagonists. It has also been suggested that P450 IIIA is a major subfamily involved in aldrin epoxidation in man (1). Use of nifedipine as a routine probe for P450 IIIA is complicated by its photosensitivity. We therefore determined the rate of felodipine metabolism by measuring the formation of the primary metabolite, dehydrofelodipine, and also aldrin epoxidation in 23 human liver microsomal samples. Activities showed 10-fold variation in rates of felodipine oxidation and 5-fold variation in aldrin epoxidation between livers. A unimodal distribution was observed and oxidation of felodipine correlated with aldrin epoxidation ($r=0.89, p<0.01$) suggesting that common P450 isozymes are involved. Immmunoinhibition studies were carried out using a polyclonal antibody raised against a purified human P450 IIIA protein. The antibody has been previously shown to inhibit cyclosporin A metabolism (2), yet the antibody did not inhibit either felodipine oxidation or aldrin epoxidation. Western Blot analysis revealed that immmunochemically determined concentrations of P450 IIIA correlated with felodipine oxidation activities. Results obtained confirm that P450 IIIA is the principal subfamily responsible for the metabolism of felodipine and suggest that cyclosporin A and felodipine are metabolised by similar, but distinct, members of this subfamily. Felodipine oxidation may be a useful in vitro probe for P450 IIIA activity in man.

1 Guengerich F P et al J Biol Chem 261,5051-5060,1986.
2 Shaw P M et al Biochem J 263,653-663,1989.

IN VITRO METHODOLOGIES; METABOLISM OF NICOTINE IN EPIDERMAL CULTURES OF THE NEW-BORN RAT

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Utilization of cell culture to evaluate the metabolism and toxicity of drugs may be considered to be a useful *in vitro* model. Recently we have investigated the metabolism of nicotine in epidermal cell cultures of new-born rat, and compared the resulting metabolite profile with that in isolated hepatocyte(IH) and hepatic microsome(MS) of rat and monkey. In this study we also have checked leakage of enzymes from cells as an index of toxicity. The major metabolite of nicotine in epidermal cell culture system was cotinine; a dehydrogenated metabolite was also detected and identified by NMR and Mass spectroscopy. Similar metabolites were observed in IH and MS system and thus showed that epidermal cell culture is a useful model of *in vitro* metabolism. The *in vivo* metabolic profile of nicotine, resulting from its dermal application, will also be discussed.

IDENTIFICATION OF THE MAJOR METABOLITES OF [^{3}H]-(+)-8-CHLORO-5-(2,3-DIHYDROBENZUFURAN-7-YL)-7-METHOXYMETHYLOXY-3-METHYL-2,3,4,5-TETRAHYDRO-1H-3-BENZAZEPINE (NNC 01-1006) IN RATS.

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Six metabolites of NNC 01-1006 were isolated from rat urine and their structure determined by NMR and/or MS. Metabolites were formed by N-demethylation, hydroxylation of the dihydrobenzofuran-moity, or elemination of the methoxymethyl-group followed by glucuronidation. All combinations were found in urine. In feces metabolites formed by hydroxylation and elimination of the methoxymethyl-group dominated, while N-demethylated metabolites only were present in small amounts. No glucuronides were found in feces. The main plasma metabolite was NNC 01-1006 without the methoxymethyl-group (as glucuronide). Only minute amounts of other metabolites were present in plasma.

PHOTOAFFINITY LABELING OF RAT CYTOCHROME P4501A1 BY AZIDOWARFARINS

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The substrate binding site amino acid compositions of P450s, and their role in P450 substrate specificities, are unknown. Photoaffinity labeling provides a powerful tool for determination of these residues. Based on the sites of P450 catalyzed hydroxylation of the substrate warfarin, three photoprobes (4'-, 6-, and 7-azidowarfarin) have been designed and synthesized. Aqueous solutions of these photoprobes at room temperature exhibited variable stability to ambient light - 4'-azidowarfarin was indefinitely stable, 6-azidowarfarin decomposed ~30% after 1 hr, and 7-azidowarfarin completely decomposed in neutral solution within minutes but was stable in strongly acidic solutions (pH <2). All three exhibit light-dependent (254 nm, at -196°C) inactivation of subsequently reconstituted P-4501A1 catalyzed metabolism of warfarin to 6- and 8-hydroxywarfarin. P4501A1 (2.5 μM) was inhibited 50% by photoactivated 4'-, 6-, and 7-azidowarfarin at 161, 4.8 and 2.8 μM, respectively. Photolysis of the probes prior to their addition to P4501A1 produced substantially less inhibition. The 4'-azidowarfarin inhibition of P4501A1 was prevented by coincubation with excess (R)-warfarin, but with 6-azidowarfarin the affinity is so high that (R)-warfarin did not prevent photoinactivation of P4501A1. Current studies are aimed at the identification of P4501A1 substrate binding site amino acids utilizing tritium labeled photoprobes. (Partially funded by the American Cancer Society).

GSH-INDEPENDENT DENITRATION OF CD-349, A NITRATE ESTER OF DIHYDROPYRIDINE DERIVATIVE, BY RABBIT HEPATIC CYTOSOL

M.Ogawa, K.Mizuno, H.Itoga, K.Fukushima, T.Suwa, T.Igarashi*, and T.Satoh*. Research Center, Taisho Pharmaceutical Co.,Ltd., Ohmiya, Japan. *Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan

It has been widely accepted that the metabolic denitration of organic nitrate esters such as nitroglycerin and isosorbide dinitrate, is catalized by glutathione S-transferase(GST). We investigated the denitration of CD-349, a dihydropyridine derivative having two nitrate esters, by rabbit hepatic cytosol. The denitration activity of dialyzed cytosol was potentiated by addition of GSH and more markedly by dithiothreitol(DTT). Cytosolic fraction after ammonium sulfate precipitation(30-60%) was applied to Sephadex G-150 chromatography, and two distinct peaks, named peak I and peak II were obtained. They possessed denitration activity of CD-349. The activity of peak I was activated by the thiol compounds such as dihydrolipoamide and DTT, but not by GSH. Moreover, it was not inhibited by S-hexyl GSH, a inhibitor of GST, indicating peak I possessed no GST activity. In contrast, the denitration activity of peak II having GST activity required GSH and was inhibited by S-hexyl GSH. And, both the denitration and GST activities of peak II were not affected by dihydrolipoamide and DTT. These results strongly suggested that the GSH-independent enzyme(peak I) in addition to GST(peak II), was also found to be responsible for denitration of nitrate esters.

EFFECTS OF ENZYME INDUCERS AND INHIBITORS ON THE IN VITRO MICROSOMAL N-OXIDATION OF 2-AMINO-1-BENZYLBENZIMIDAZOLE

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The metabolism of 2-amino-1-benzylbenzimidazole (ABB) was undertaken using hepatic microsomes from various species to investigate whether an N-oxide or N-hydroxylated compound was formed. N-Hydroxylation of the 2-amino group was observed using microsomes from all species studied, the greatest activity being found in the hamster followed by mice, rat, rabbit and guinea-pig. N-Debenzylation was observed in all species; N-oxide formation was not observed.

The formation of the N-hydroxy metabolite was assayed using HPLC and UV-RAPISCAN monitor. The enzymic N-hydroxylation was enhanced by pretreatment of hamsters by β-naphthoflavone and to a lesser extent by phenobarbitone. N-Hydroxylation was inhibited by SKF525A, DPEA, methimazole, metyrapone.

The present evidence strongly support the involvement of Cytochrome P_{448} in the microsomal N-hydroxylation of ABB. It is suggested that ABB may be a model substrate mimicing the complex mutagenic aminoimidazoles formed during the pyrolysis of proteins and amino acids.

MECHANISM OF SLOW RELEASING OF HALOPERIDOL AFTER INTRAMUSCULAR INJECTION OF HALOPERIDOL DECANOATE

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Intramuscular injection of haloperidol decanoate (HD), an ester of haloperidol (H) and decanoic acid, results in a sustained plasma level of pharmacologically active principle, H, for a period as long as about one month. Present studies concern the pharmacokinetics of HD for clarifying the mechanism of slow releasing of H.

^{14}C-labeled HD and H were injected intravenously or intramuscularly to rats. Plasma levels of HD and H were determined by TLC-radiochromatography and fitted to compartment model for their rate constants.

After intravenous injection of H, H in plasma decreased with elimination rate constant of about 20 day^{-1}. Plasma levels of HD injected intravenously resulted in the rate constant of about 2 day^{-1}. The constant of HD conceivably reflects that of *in vivo* hydrolysis of HD to H, because HD was excreted from the body only after conversion to metabolites of H. After intramuscular injection of HD, plasma levels of HD and H decreased with rate constants of about 0.05 and 0.07 day^{-1} (half lives of about 15 and 10 days), respectively. The absorption rate constant was estimated to be about 0.05 day^{-1}. This indicates that the absorption of HD was a rate-limiting step of HD disposition after intramuscular injection, namely a flip-flop disposition. Levels of HD in the regional lymph nodes in the injection site were the highest in examined lymph nodes and plasma, suggesting the absorption of this ester via lymphatic system. The slow releasing of H after intramuscular injection of HD can be thus attributable to its flip-flop kinetics based on slow absorption via lymphatic system from the injected site.

DIFFERENT METABOLIC ACTIVITIES OF EXPRESSED ENZYMES OF TWO P-450IIC9 cDNA CLONES

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Since results of clinical studies have demonstrated that there were individuals having poor capacity to metabolize mephenytoin and tolbutamide, genetic polymorphisms on forms of cytochrome P-450 related to these metabolisms have been focused on the basis of gene structure. We have isolated two P-450IIC9 cDNA clones from the same human liver cDNA library. Nucleotide sequence of one of isolated clones (hPA22) was the same as that of the other P-450IIC9 cDNA clones reported, pHY13 and MP-4. However, the other isolated clone (hPA6) deleted 6 bases near the middle in the clone as compared with hPA22. To clarify the effect of the 6 base-deletion on the metabolic activity of the encoded protein, both clones were inserted into yeast expression vector and yeast were transformed by the constructed expression plasmids. Expression of both clones were demonstrated by Northern blot analysis and Western blot analysis using antibodies against P-450-HM2, a possibly identical P-450IIC protein. The tolbutamide methylhydroxylase activities of these expressed enzymes were measured; the activity of the expressed enzyme encoded by hPA6 was lower than that encoded by hPA22. Thus, the 2 amino acid-deletion resulting from the 6 base-deletion reduced the enzymatic activity.

RADIOLUMINOGRAPHY FOR QUANTITATIVE AUTORADIOGRAPHY OF ^{14}C AND ^{125}I

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A new combination of new photosensitive material, so called Imaging Plate (IP) and a new computed Imaging Analyzer System is presented, hereupon, as a new term of "Radioluminography". Displayed images by Radioluminography can give completely quantitative data for any kind of particle emitters of radioactive nuclides as shown by our previous presentation. The most characteristic specificities of "Radioluminography" are caused by its most accurate quantitativeness, its highest sensitivity, and its most skillful computed functions than ever before. A linear relationship between ^{14}C- radioactivity and PSL(photo stimulated luminescence) intensity is shown clearly under the condition of radioactive concentration of 2×10^4 to 2×10^4 pCi for 18 hr. exposure and 2×10^4 to 2×10^6 pCi for 0.25 hr. exposure. The highest sensitivity is shown by 20 pCi for 18 hr. exposure.
In case of ^{125}I, radioactivity of 10^2 to 10^4 pCi for 9hr. exposure and 10^3 to 10^5 pCi for 2hr. exposure, respectively indicated a good linearity. The highest sensitivity for ^{125}I is shown by 10 pCi for 9 hr. exposure or 10^3 pCi for 0.25 hr. exposure.

IRREVERSIBLE AND ACTIVE SITE DIRECTED INHIBITION OF GLUTATHIONE S-TRANSFERASES

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Quinones are known for their reactivity towards sulfhydryl groups, and thus are potential protein alkylating compounds. Normally, quinones are detoxified by conjugation with glutathione. Some conjugates retain their oxidized nature after conjugation (e.g. halogen substituted quinones), while others have been shown to undergo intracellular oxidation. Thus, glutathione conjugates of quinones are formed, which display a special type of reactivity, i.e. selective inhibition of glutathione S-transferase isoenzymes (GST).

Irreversible inhibitors of GST were developed, combining chemical reactivity with a high degree of selectivity for the active site of GST. For example, the glutathione conjugate of tetrachloro-1,4-benzoquinone (GS-TCBQ) effectively reacts with a sulfhydryl group in or in the vicinity of the GST active site. The glutathione moiety acts as a "targeting" component. GS-TCBQ irreversibly inhibits the catalytic activity of almost all rat and human GST isoenzymes for approximately 80% at equimolar concentrations of inhibitor and enzyme, independent of the enzyme concentration. The only exception is the human isoenzyme B2-B2, which contains no cysteine residues. At 0°C, the inhibition is achieved within 10-100 seconds, depending on the isoenzyme used. Competitive inhibitors of GST slow down but ultimately do not prevent inactivation by GS-TCBQ. The mercaptoethanol conjugate of TCBQ, which chemically behaves identically to GS-TCBQ, inhibits GST at a more than 20-fold lower rate, stressing the strong targeting effect of the glutathione part.

TCBQ and GS-TCBQ proved to be useful tools in the study of the GST active site. Both spectral quantitation experiments and radioactive labeling experiments indicate complete loss of catalytic activity after modification of only one cysteine residue. The location of this cysteine residue was determined by identification of the TCBQ labeled peptide fragment after CNBr-cleavage.

Structure activity relationships using various chlorinated benzoquinones and naphthoquinones and their glutathione conjugates were investigated with rat and human isoenzymes, giving insight both in useful quinones and in isoenzyme selectivity.

A large number of naturally occuring hydroquinones may cause inhibition of GST after oxidation. As an example, caffeic acid irreveribly inhibits GST isoenzymes after spontaneous oxidation. Depending on the isoenzyme, this coincides with covalent dimerisation of the two GST subunits.

These results may lead to the development of isoenzyme selective GST inhibitors, with applications in drug metabolism research and drug resistance.

ESTABLISHMENT OF AN EVOLUTIONARY CONSERVED MICROSOMAL CLASS OF LIVER ENZYMES INVOLVED IN STEROIDOGENESIS AND REDUCTIVE XENOBIOTIC CARBONYL METABOLISM

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Carbonyl group containing compounds are widely distributed in the organism and appear as steroid hormones, mediators, transmitters, intermediates and xenobiotics. The reductive biotransformation to the respective alcohol metabolite is carried out by oxidoreductases localized in cytosol and/or microsomal fractions of the cell.

Using the diagnostic cytochrome P450 inhibitor metyrapone as a model substrate for reductive xenobiotic carbonyl metabolism it is revealed that a substantial part of the microsomal carbonyl reduction in various mammalian liver sources is catalyzed by a family of related enzymes which share structural and functional properties. While exhibiting 3α-OH-steroid dehydrogenase and carbonyl reductase activity they participate in the inactivation of androgens as shown by purified enzymes and/or inhibition of microsomal carbonyl reduction by androsterone or 5α-Dihydrotestosterone. Immunoblotting of liver microsomes prepared from the examined species reveals strong and specific crossreactions with antibodies against purified microsomal mouse liver enzyme.

The structural relationship to a procaryontic 3α-OH-steroid dehydrogenase from Pseudomonas testosteroni and to a protein from the insect species Calliphora vicina are revealed by Western Blot analysis. Both have high specific activities for carbonyl reduction and can be strongly inhibited by ecdysteroids.

Flutamid and medroxyprogesterone acetate inhibit the respective human enzyme which is expressed in adult liver of both sexes.

It is concluded that a great part of the overall found ability for carbonyl reduction in mammalian liver microsomes can be attributed to an evolutionary conserved class of proteins involved in steroidogenesis.

CONJUGATION PATHWAYS OF HUMAN LIVER: A STUDY ON THE INTERINDIVIDUAL VARIABILITY.

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Little is known on the interindividual variability for conjugation reactions and we measured the activities of 11 conjugation enzymes in 100 human liver specimens. Wedge liver specimens, obtained from 58 women and 42 men aged between 25 and 70 years undergoing cholecistectomy, were stored at -80° C for up to 3 years. The activities of the following enzyme were studied: Phenol-sulphotransferase (PST, 4 uM p-nitrophenol), catechol-sulphotransferase (CST, 60 uM dopamine), desipramine N-sulphotransferse (D-NST, 1 mM desipramine), minoxidil sulphotransferase (MST, 14.7 mM minoxidil), glutathione S-transferase (GST, 1 mM 3,4-dichloro-1-nitrobenzene), thiopurinemethiltransferase (TPMT, 4 mM 6-mercaptopurine), thiomethiltransferase (TMT, 5 mM captopril), glycinetransferase (Gly-T, 0.2 mM benzoic acid), histamine N-methyltransferase (H-NMT, 0.3 mM histamine), morphine glucuronyltransferase (M-GT, 1.5 mM morphine), 2-naphthol glucuronyltransferase (NT-GT, 0.5 mM 2-naphthol). The variation coefficient (VC) ranged between 29% (H-NMT) and 130% (Gly-T). The frequency distribution histogram was significantly normal for H-NMT and GST whose VC was about 30%, whereas a positively skewed histogram was obtained for the other enzymes. The probit analysis was compatible with the presence of only one group in the population for all the enzymes studied. All possible correlation between enzyme activities were tested and those significant were: PST and H-NMT ($P<0.05$), PST and Gly-T ($P<0.01$), PST and MST ($P<0.001$), M-GT and D-NST ($P<0.01$), GST and H-NMT ($P<0.01$), GST and MST ($P<0.01$), GST and M-GT ($P<0.01$), GST and PST ($P<0.01$), TMT and Gly-T ($P<0.05$) and TMT and TPMT ($P<0.05$). This work shows that interindividual variability is enzyme dependent in human liver. Correlations between enzyme activities might suggest that different enzymes share common control.

SULPHATION AND GLUCURONIDATION OF RITODRINE IN FETAL AND ADULT HUMAN TISSUES.

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Ritodrine (R), a Beta-2 agonist, is used for the management of the preterm labor. Excretion of R-sulphate is similar to R-glucuronide in maternal urine whereas, R-sulphate is prevalent over R-glucuronide in the urine of the newborn infant (Brashear et al., Clin. Pharmacol. Ther. 44, 634, 1988). These findings might suggest that sulphation is prevalent over glucuronidation in fetal life but not in adulthood. To verify this hypothesis, we measured the rates of R-sulphation and R-glucuronidation in human fetal and adult tissues. Two radiometric assays, one for R-sulphation and the other for R-glucuronidation, were developed. Average (pmol/min/mg ± SD) rate of R-sulphation was 308±338 (mid-gestation fetal liver, N = 48) and 196±73 (adult liver, N = 27). The rate of R-sulphation correlated with those of dopamine-sulphation and p-nitrophenol- sulphation in both fetal and adult livers. Michaelis-Menten constant (average ± SD) for ST with R as substrate was 3.84±0.50 mM (fetal liver, N = 4) and 9.45±3.4 mM (adult liver, N = 4). Fetal and adult lung kidney and gut catalyzed the R-sulphation, and gut was more active than liver. R-Glucuronidation was catalyzed by adult livers, the average (±SD) rate being 523±144 pmol/min/mg, whereas only 5 of the 48 fetal livers assayed were active in the formation of R-glucuronide and the average (±SD) rate in the active livers was 44.6±34.8 pmol/min/mg. This work corroborate our postulate thus, R-sulphation is better developed than R-glucuronidation in the human fetus.

PHARMACOKINETIC CHARACTERISTICS OF HYDROXYLATED METABOLITES OF MEXILETINE

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The metabolism of mexiletine (MEX), antiarrhythmic agent, has been reported to proceed mainly via hydroxylation followed by secondary methylation, deamination, reduction and conjugation. The objective of this study was to investigate plasma pharmacokinetics of series of hydroxylated metabolites of MEX: hydroxymethyl-MEX (OHMeMEX), p-hydroxy-MEX (p-OHMEX), its corresponding alcohols (OHMeMEX-OL, p-OHMEX-OL) and N-hydroxy-MEX (N-OHMEX).

Methods: 10 male rabbits each received orally 5 mg/kg MEX with subsequent collection of plasma samples. Plasma concentrations of MEX and its metabolites were determined by specific HPLC method and confirmed by mass spectrometry.

Results: Mean values

Metabolite	t_{max} (h)	C_{max} (µg/mL)	AUC^{t}_{0} (µg h/mL)	$t_{0.5}$ (h)	R_m
OHMeMEX	2.0	0.179	0.66	2.4	0.26
p-OHMEX	2.3	0.059	0.36	2.1	0.14
OHMeMEX-OL	2.0	0.057	0.34	1.5	0.13
N-OHMEX	3.4	0.082	0.45	1.8	0.18

R_m - metabolic ratio: AUC_{total}(met) / AUC_{total}(drug)
No plasma levels of p-OHMEX-OL were detected

Conclusions: The oxidative metabolism of MEX occurs mainly via aliphatic hydroxylation. Further investigations of urinary excretion of these metabolites will be necessary.

Depletion of Erythrocyte Glutathione by Extracellularly Activated Metabolites.

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Determination of glutathione (GSH) depletion as it occurs after *in vitro* metabolic activation by liver microsomes, forms an adequate method for the evaluation of reactive metabolite formation (1). GSH depletion occurs also in humans for instance after exposure to the soil fumigant dichloropropene (2). *In vivo* metabolite formation does not necessarily occur within the same cell (i.e. the erythrocyte) where GSH depletion is found. We used an *in vitro* system consisting of rat liver microsomes and human erythrocytes, to study the ability of the formed metabolites to enter the erythrocyte. Addition of 1 mM 3-acetamidophenol (3AcAP), cyclophosphamide (CP) or bromobenzene (BrB) to this system lead to over 50 % depletion in erythrocytes (20 fold diluted suspension). All 3 substances did also deplete GSH which was directly added to the microsomes. Depletion did not occur when the substances were added to erythrocytes in the absence of microsomes. This indicates that reactive metabolites of 3AcAP, CP and BrB originated from microsomal activation were able to enter the erythrocyte.

[1] Reactive metabolite formation in fortified liver microsomes. Invittox protocol **10**, Nottingham 1989.
[2] Brouwer E.J., Evelo C.T.A., Verplanke A.J.W. van Welie R.T.H. and de Wolff F.A. (1991) Biological effect monitoring of occupational exposure to 1,3-dichloropropene: effects on liver and renal function and on glutathione conjugation. *Br. J. Ind. Med.* **48**: 167-172.

SPECIES DIFFERENCE IN METABOLISM OF GYKI-52 322, A NEW PSYCHOACTIVE 5H-2,3-BENZODIAZEPINE

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GYKI-52 322 /1-(4-aminophenyl)-4-methyl-7,8-dimethoxy-5H-2,3-benzodiazepine/ has shown atypical neuroleptic and anxiolytic activity in animal experiments (1). Rats and dogs of both sexes were treated with ^{14}C-labelled compound at a dose of 10 mg/kg orally. Metabolic profiles were investigated in serum, urine, bile and feces. Radioactive components were isolated and purified with TLC. The structures of the metabolites were identified by mass spectrometry followed by comparison of their chromatographic properties with those of the synthetized compounds.

Peak serum radioactivity levels were measured 45 min. and 3h in rats and dogs, respectively. As the main components in the serum N-acetyl-52 322 could be detected in the rat and GYKI-52 322 in the dogs.

Rats eliminated the radioactive substance rapidly both in the urine and feces. About half of the metabolites excreted in the urine were in free form, the remainder was conjugated. The high proportion of radioactivity found in feces samples is supposed to be due to intense biliary excretion. The original compound was detected in feces in trace amounts. Dogs eliminated the substance mostly in urine without notable conjugation. The original compound gave rise to about half of radioactivity of urine samples. In rats a very rapid and intense acetylation took place, resulting in the N-acetyl derivative. A part of this metabolite was further transformed mainly by O-demethylation followed by conjugation at the hydroxyl group. In dogs the amino group was not affected, the main metabolic pathway of GYKI-52 322 was found to be O-demethylation.

1. Horváth et al: Arzneim.-Forsch. 39 , 894-899 /1989/

THE ENZYMOLOGY INVOLVED IN THE IN VITRO METABOLISM OF MIFENTIDINE

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Studies on mifentidine, a novel histamine H_2-receptor antagonist, have shown this compound to be an effective ulcer-treating and ulcer-preventing agent. The in vitro metabolism of mifentidine in different species has previously been studied by us (Pattichis et al., J. Pharmacol. Clin. Pharmacol., in press; Kajbaf et al., Anal. Chim. Acta, in press). In the present study, we attempted to elucidate the enzymology involved in the in vitro metabolism of mifentidine in rabbits. After treatment of the animals with potential enzyme inducers, the microsomal biotransformation of mifentidine to its main metabolites was investigated and compared to controls. The effect of enzyme inhibitors was also studied in vitro. Pretreatment with phenobarbitone and pyridine increased the conversion of mifentidine to 4-imidazolylphenylamine (DA 4036), imidazolylphenylformamide (DA 4634), the urea derivative of mifentidine (DA 6075) and the imidazole-hydroxylated derivative of 4036 (DA 6035). SKF525A, N-octylamine and DPEA decreased the conversion of mifentidine to DA 4036, DA 4634, DA 6075 and DA 6035. These results indicate the involvement of cytochrome P450 in the in vitro biotransformation of mifentidine.

COUMARIN 7-HYDROXYLATION BY HUMAN LIVER MICROSOMES: PRONOUNCED INTER-INDIVIDUAL DIFFERENCES REFLECT DIFFERENCES IN P-450 IIA3 LEVELS.

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Rat IIA1 and IIA2 are two closely related liver microsomal P-450 enzymes that catalyze the 7α- and 15α- hydroxylation of testosterone, respectively. Antibody against rat IIA1, which cross reacts strongly with IIA2, recognizes a single protein in human liver microsomes. However, human liver microsomes do not catalyze the 7α- and 15α- hydroxylation of testosterone. The results of the present study suggest that the protein in human liver microsomes recognized by *anti*-IIA1 is cytochrome P-450 IIA3, and that this enzyme is responsible for catalyzing the 7-hydroxylation of coumarin. The rate of coumarin 7-hydroxylation varied ~17 fold among liver microsomes from 22 human liver samples (280-4750 pmol/mg protein/min). This variation coincided with inter-individual differences in the levels of IIA3, as determined by immunoblotting. *Anti*-IIA1 completely inhibited (>95%) the 7-hydroxylation of coumarin by human liver microsomes. Proportionately more antibody was required to inhibit those microsomal samples with high coumarin 7-hydroxylase activity than those with low activity. A single high affinity enzyme (Km ~0.5 μM) catalyzed the 7-hydroxylation of coumarin, regardless of the rate of coumarin 7-hydroxylation. Consequently, inter-individual differences in coumarin 7-hydroxylase activity were observed over a wide range of substrate concentrations (0.1 to 1,000 μM). Interestingly, just as human liver microsomes do not appear to contain IIA1 or IIA2, it does not appear that rat liver microsomes contain IIA3, inasmuch as rat liver microsomes do not catalyze the 7-hydroxylation of coumarin (<20 pmol/mg protein/min). These results suggest that coumarin 7-hydroxylase activity is a reliable marker for IIA3 levels in human liver microsomes, and that the level of this P-450 enzyme shows considerable inter-individual variation.

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SUBCELLULAR DISTRIBUTION OF GLUTATHIONE-RELATED ENZYMES IN HUMAN FETAL ADRENAL GLAND Ren-Xiu Peng, Yu-Shan Wang* and Shao-Bo Lai* Department of Pharmacology, Hubei Medical College Wuhan, 430071, China

Glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), and glutathione reductase(GR) activities were investigated in different centrifugal fractions of human fetal adrenal glands. Fetuses were obtained from Department of Gynaecology where legal abortions were performed. GST in adrenal microsomes was 112 ± 34 nmol·min^{-1}·mg^{-1} protein, exhibited 8 folds higher than fetal liver microsomes reported previously, when using CDNB as substrate. GST activity was also mainly detected in 105,000 x g supernatant and S_9, and the values were 191 ± 89, 147 ± 84 nmol·min^{-1}·mg^{-1}, respectively, nevertheless, comparatively low in mitochondria(62 ± 35 nmol·min^{-1}·mg^{-1}). Since all experiments were simutaneously performed with adrenal and liver in each case, the comparable results indicated that GST in all different fractions of adrenal studied were markedly higher, being 1.7-2.7 folds, of fetal liver values. In S_9, GSH-Px may amount to as much as 50 % of the mitochondria fraction, the value was 15 ± 7 nmol·min^{-1}·mg^{-1}. Little or no GSH-Px activity could be detected in corresponding part of fetal liver. Controversely, GR was mainly in S_9. The present results suggest that comparatively high GSH-related enzymatic activities in adrenal may play an important role in human fetal Pharmacology and Toxicology.

IDENTIFICATION OF THE MAIN METABOLITES OF 2-ETHYLHEXANOIC ACID IN RAT URINE USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The metabolites of 2-ethylhexanoic acid, an industrial chemical and the active ingredient in a wood preservative, were investigated in rat urine. Male Wistar rats were given 2-ethylhexanoic acid (2-EHA) in drinking water (600mg/kg daily) for nine weeks. At the end of that period urine specimens were collected. The compounds were identified by gas chromatograph-mass spectrometry using the electron impact ionization and chemical ionization techniques. In addition to 2-EHA, ten different 2-EHA-related metabolites were identified in the urine of the 2-EHA-treated rats. The main metabolite was 2-ethyl-1,6-hexanedioic acid. Moreover, 2-ethyl-6-hydroxyhexanoic acid was found and five other hydroxylated metabolites and two lactones, the detailed structure of which have not yet been analyzed. Urine contained also unsaturated 5,6-dehydro-EHA. 5,6-dehydro-EHA is the metabolite corresponding to 2-n-propyl-4-pentenoic acid, the hepatotoxic metabolite of valproic acid. At least part of the 2-EHA is present in urine as a glucuronide conjugate.

PRELIMINARY PHARMACOKINETIC DATA ON THE IRREVERSIBLE AROMATASE INHIBITOR FCE 24304 IN POSTMENOPAUSAL WOMEN

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FCE 24304 (6-methylenandrosta-1,4-diene-3,17-dione) is an irreversible aromatase inhibitor capable of reducing aromatase activity both *in vitro* and *in vivo*. The compound showed no interference with rat prostatic 5α-reductase activity and no inhibition of rat adrenal desmolase. The present study was carried out in order to obtain preliminary pharmacokinetic data of the compound in humans. 12 healthy postmenopausal volunteers aged between 49 and 76 years participated in the study. The volunteers (3/dose) received single oral doses of 50, 200, 400 and 800 mg FCE 24304 within 15-30 min after a fat-rich breakfast. Blood samples were drawn at 0.5, 1, 2, 4, 8, 24 and 48 h after administration. Plasma was assayed by HPLC, with a limit of detection of 10 ng/ml. FCE 24304 was rapidly absorbed and peak plasma levels of 27±6, 221±33, 343±83 and 414±77 ng/ml (mean values±S.E.M.) were reached within 2 h after doses of 50, 200, 400 and 800 mg, respectively. Plasma concentra- tions declined rapidly thereafter and fell below 10 ng/ml 24 h after the doses of 400 and 200 mg, whereas after the 50 mg dose, non-measurable plasma levels were already found 4 h after administration. Mean plasma AUC_{0-8h} values were 566±104, 907±222 and 1081±105 ng.h/ml (mean±S.E.M.) after 200, 400 and 800 mg, respectively. These preliminary data show a dose-related increase of plasma levels of FCE 24304 following oral doses up to 400 mg.

Metabolism of cinnamaldehyde in male F344 rat and rat suspended hepatocytes

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trans-Cinnamaldehyde (CA) is used extensively in both the perfume and flavour industries. CA undergoes oxidation to cinnamic acid (CAC) and reversible reduction to cinnamic alcohol (CALC), but can also (like other α,β- unsaturated compounds) react with reduced glutathione (GSH). We have examined the relative importance of these two elimination pathways as a function of dose size in the rat and in suspended rat hepatocytes.

Male F344 rats were given a single *i.p.* dose of 1.7 or 250 mg/kg ^{14}C-CA in corn oil. Urine and faeces were collected for 3 days, assayed for radioactivity and analysed by radio HPLC. Hepatocytes in suspension (10^6 cells/ml) were treated with ^{14}C-CA or DMSO. Samples were taken for radio HPLC, binding of radiolabel to cellular macromolecules, GSH depletion and lactate dehydrogenase (LDH) leakage.

Elimination of CA in the rat is rapid. Of the 250 mg/kg dose 84.21 ± 5.05% (n=4; ± se) was excreted in the urine, 0.79 ± 0.49% in the faeces in the first 24 hrs. Total recovery after 72 hrs was 102.00 ± 5.10%. Hippuric acid was the major urinary metabolite (83.09 ± 2.13%). Intermediates of the oxidation pathway accounted for 6% and sulphur containing metabolites 8%. No significant change was observed in excretion or metabolic profile at the 1.7 mg/kg dose. In hepatocyte suspension 5×10^{-4}M CA is rapidly metabolised to CAC and subsequently to benzoic acid and hippuric acid. CALC is present in the first hour. 10^{-4}M and 5×10^{-4}M CA were fully metabolised within 1 hr. At CA concentrations of 10^{-3} M and higher, its metabolism was saturated and the parent compound remained present, with concomitant enhanced macromolecular binding, GSH depletion and cytotoxicity (LDH leakage).

We conclude that CA toxicity observed *in vitro* is due to the parent compound *per se* and the rate limiting step in its elimination is the formation of CAC. The *in vivo* metabolic profile does not change significantly over the dose range 1.7 - 250 mg/kg suggesting that *in vivo* liver levels do not exceed the threshold value of 10^{-3}M. Present studies are being undertaken to confirm this.

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INDIVIDUALIZATION OF THERAPY - THE PROBLEM OF SUSTAINED RELEASE CARBAMAZEPINE PREPARATION

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In order to solve the problem arising in individualization of therapy with antiepileptic drugs, the pharmacokinetic parameters obtained for the first domestic sustained release carbamazepine (CBZ) preparation were used to predict the body accumulation of CBZ following the dosage regimen suggested by the manufacturer.

Due to slow absorption and enteral reabsorption, as well as interindividual metabolic enzyme (MFO) variability influencing the elimination of CBZ, it could be assumed that the dosage regimen suggested by the manufacturer (2 x 400 mg CBZ per day) would not be appropriate for the entire population.

Therefore, a new computer program was generated; it was used to determine the optimal drug dose and dosing intervals utilizing three CBZ blood concentrations (before the dose, and 48 h and 96 h post-dose) determined in patients.

In conclusion, administration of sustained release CBZ preparations does not exclude the need of frequent drug blood level determination, as the interindividual variations in general population are of greater importance when sustained release formulations are administered in comparison with the conventional ones.

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STEREOINVERSION OF α-BROMOCARBOXYLIC ACIDS IN THE RAT IN VIVO.

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GSH conjugation of the BIU (α-bromoisovalerylurea) enantiomers is stereoselective: (R)-BIU is conjugated faster than (S)-BIU. When the urea moiety was omitted, resulting in BI (α-bromoisovaleric acid), GSH conjugation was stereospecific: only (S)-BI was conjugated. In vivo, stereoinversion of (R)-BI gave rise to (S)-BI that could be conjugated (1). In the present work, the four diastereomers of α-bromo-3-methylvaleric acid (α-B3MV, containing two chiral centers) and their urea derivatives (α-B3MVU) have been synthetized to study whether the GSH conjugation of those α-bromocarboxylic acids and their urea derivatives is stereoselective and whether in vivo stereoinversion of the α-bromocarboxylic acids can be found.
The results show that the α-carbon of the diastereomeric α-bromocarboxylic acids had to be present in the S-configuration in order to get GSH conjugation. Furthermore, conjugation of (R,S)-α-B3MV was negligible and that of (R,R)-α-B3MV zero. The two latter compounds were inverted to the corresponding diastereomers: (S,R) and (S,S)-α-B3MV, resp., which were found to be conjugated. All urea derivatives were conjugated with GSH.
In conclusion: for the diastereomers of α-bromo-3-methylvaleric acid that showed (almost) no GSH conjugation, stereoinversion of the chiral α-carbon was observed in vivo. This was not observed for the other two diastereomeric acids, most likely because their GSH conjugation is too fast to detect any stereoinversion.

(1) Polhuijs, M et al, Biochem. Pharmacol. 38: 3957-3962, 1989.

EFFECTS OF COBALT AND MANGANESE IN LIVER AND KIDNEY OF RAT

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Cobalt and manganese are essential trace elements and play vital role in biological reactions. However, the deficiency as well as excess of these elements was reported harmful to animals and plants by many scientists. We have recorded hazardous effects of these metal ions on liver and kidney of rat as necrosis of the cell, Binucleated and multi-nucleated cells, true polycythemia, nuclear pycnosis, cell proliferations, fibro-proliferation, glomerular swelling and injured Proximal and Distal Convoluted cells. The effects of these elements on RNA, DNA and on some key enzymes were also recorded and discussed.

PREPARATION OF MICROSOMES FROM HUMAN MYOMETRIUM AND UTERINE LEIOMYOMA

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The most extensively studied methods to prepare microsomes are developed for the liver. In general the same procedures are used to isolate microsomes from extrahepatic tissues. However these tissues contain different cell types which probable do not behave similary as liver upon homogenization and centrifugation.

In this study we compared three methods to prepare microsomes from myometrium and uterine leiomyoma to measure cytochrome P450IA1-dependent aryl hydrocarbon hydroxylase (AHH) activity.

Method I: Reverse ordered (105.000g-->13.000g) differentials centrifugation produced microsomes with specific AHH-activity (fmol/min/mg prot.) 11.0 ±9.0 in myometrium and 21.0 ±17.0 in leiomyoma with average protein content 2.5mg/ml microsomal suspension (7.5mg/g tissue).

Method II: Microsomes isolated as above combined with Sepharose CL-2B gel-filtration had AHH-activities 47.0 ±42.0 and 87.0 ±67.0 respectively with protein content 0.4mg/ml (1.2mg/g tissue).

Method III: In microsomes isolated by conventionaly ordered centrifugation the values of AHH-activity were 8.0 ±9.0 and 19.0 ±10.0 with protein 0.3mg/ml (0.9mg/g tissue).

In electron micrographs the final microsomal suspensions contained the vesicles of smooth endoplasmic reticulum contaminated by few collagen fibers.

Method II is our choice to prepare uterine tissue microsomes for AHH-assays.

STIMULATION OF MONOOXYGENATION AND CONJUGATION FOLLOWING RAT LIVER TRANSPLANTATION: INVOLVEMENT OF KUPFFER CELLS.

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The success rate of liver transplantation has improved markedly during the last few years due to standardized surgical techniques and improved immunosuppressive regimens. Although this patient population receives multiple drug therapies, the effect of liver transplantation on drug metabolism has not been evaluated. Therefore, the purpose of this study was to assess drug metabolism following liver transplantation in the rat. Livers from fed Sprague-Dawley rats (180-250g) were stored in cold Euro-Collins solution, transplanted orthotopically, then perfused two hours later with oxygenated Krebs-Henseleit buffer using a non-recirculating system. Rates of p-nitroanisole O-demethylation, conjugation of p-nitrophenol and oxygen uptake were measured. All parameters studied were elevated nearly two-fold by transplantation. Specifically, monooxygenation was increased from 2.5 $\pm$ 0.3 to 4.7 $\pm$ 0.4 μmol/g/hr, conjugation was elevated from 3.9 $\pm$ 0.3 to 7.9 $\pm$ 0.2 μmol/g/hr, and O_2 uptake was stimulated from basal values of 118 to 197 μmol/g/hr. All increases were statistically significant ($p < 0.05$; $n = 4$-5 per group). Because transplantation activates Kupffer cells under these conditions, donor rats were pretreated with the Kupffer cell toxicant, gadolinium chloride ($GdCl_3$; 10 mg/kg i.v.) 30 hrs prior to storage of liver. All parameters were reduced significantly by $GdCl_3$ treatment. Therefore, we hypothesize that activation of Kupffer cells due to liver transplantation stimulates drug metabolism, possibly by releasing eicosanoids and cytokines (ES-02759).

METABOLISM OF ZIDOVUDINE (AZT) BY HUMAN LIVER MICROSOMES : SCREENING FOR INHIBITORS

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The glucuronidation of AZT by human liver microsomes has been studied. The UDPGT activities toward zidovudine measured in 29 different microsomal fractions was slightly variable among samples (1.3 to 45 nmol/min/mg protein). The apparent Km value for AZT glucuronidation was about 5 mM. 4-hydroxybiphenyl (a substrate of $UDPGT_2$) UDPGT activity was strongly correlated ($r=0.815$, $p<0.001$) with AZT-UDPGT activity. In addition, AZT glucuronidation was inhibited by numerous substrates of the $UDPGT_2$ isoform : morphine (Ki=1.8 mM), 4-hydroxybiphenyl (Ki=0.92 mM), and ketoprofen (Ki=0.75 mM) but also oxazepam, codeine, and chloramphenicol. Bilirubin and acetaminophen did not inhibit AZT glucuronidation. These results strongly suggest the involvement of $UDPGT_2$ in AZT glucuronidation.

In order, to screen potential metabolic interactions between AZT and other drugs, 40 molecules representative of 13 different therapeutic classes were tested for inhibitors of AZT glucuronidation. Drugs detected as potential inhibitors included : quinidine, probenecid, lorazepam, verapamil, nifedipine, carbamazepine, clonazepam, amphotericin B, phenytoin, and propofol.

We can therefore anticipate that these drugs should lead to potential interaction with AZT when given in association. However, clinical studies are necessary to confirm these findings.

PRETRANSLATIONAL DEPRESSION OF CYTOCHROME P-450IA1 DURING THE ACTIVATION OF HOST DEFENCE MECHANISMS.

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When interferon is administered or produced during the activation of host defence mechanisms a loss of cytochrome P-450 and related drug biotransformation occurs in the liver. In this paper we have investigated the mechanism involved in the loss of Cytochrome P-450IA1 by interferon. The administration of the interferon inducing agent poly IC for 24 hours depressed hepatic microsomal cytochrome P-450 and ethoxyresorufin dealkylase by 30% and 47% respectively. Following 1 or 6 hours of treatment these parameters were unchanged. The levels of cyt P450IA1 mRNA were decreased by 35% and 40% 6 and 24 hours after poly IC treatment. No change in mRNA was observed after 1 hour of treatment. The total amount of mRNA present in the liver as assessed by hybridisation to a poly A oligonucleotide was not affected by poly IC treatment at any time. These experiments indicate that the interferon mediated loss of cytochrome P-450IA1 and ethoxyresorufin dealkylase results from a decrease in the synthesis of the apoprotein at a pretranslational step.

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WARFARIN HYDROXYLATION CATALYZED BY HUMAN P-450's EXPRESSED IN HepG2 CELLS

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Warfarin may be considered a model compound for investigations into the mechanism of drug interactions. One such mechanism involves inhibition of the oxidative pathway which results in (S)-7-hydroxywarfarin, the principal inactive metabolite found *in vivo*. The identity of specific human P-450 isozymes which participate in the formation of (S)-7-hydroxywarfarin was investigated. Eleven human P-450 isozymes, expressed in HepG2 cells, were examined with regard to their warfarin phenol metabolite profiles. P-450's 2A3, 2D6, 2E1 and 4B1 displayed no detectable metabolic activity towards either enantiomer of the drug. P-450 1A2 was an (R)-6-hydroxylase. P-450's 2B7 and 2F1 were (R)-4'-hydroxylases, whereas P-450 2C8 was principally an (S)-4'-hydroxylase. The major phenolic metabolite obtained from P-450 3A4 was also (S)-4'-hydroxywarfarin, however this form was principally an (R)-10-hydroxylase, as was 3A5. P-450 2C9 was the only isozyme examined which was predominantly an (S)-7-hydroxylase. 2C9 formed no detectable quantities of phenolic metabolites from (R)-warfarin. Both P-450 2C9 and three separate human liver microsomal preparations exhibited a K_m for (S)-warfarin of 4 μM. Sulfaphenazole selectively inhibited the human liver microsomal (S)-7-hydroxylase in a competitive manner with a K_i of 0.21 μM. The K_i for inhibition of 2C9-mediated metabolism of (S)-warfarin by sulfaphenazole was 0.18 μM. Therefore, we conclude that, despite the overall complexity of warfarin metabolism in humans, only those drugs that can effectively inhibit P-450 2C9 need be considered as agents that have the potential to produce clinically significant drug interactions by an inhibitory mechanism.

BIOACTIVATION OF PARA-FLUORINATED ANILINES TO BENZOQUINONEIMINES AS PRIMARY REACTION PRODUCTS

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In vivo and in vitro biotransformation of fluoroanilines was studied using ^{19}F NMR. The cytochrome P-450 dependent monooxygenation of anilines with a fluorinated para position (para with respect to the amino moiety), proceeds by formation of a fluoride anion and the reactive benzoquinoneimine as primary reaction products. Monooxygenation of fluoroanilines with a non-fluorinated para position proceeds by formation of the para-hydroxylated derivative as the primary metabolite. Thus, for fluoroanilines with a fluoro substituent at the favorable position for monooxygenation, bioactivation to the reactive benzoquinoneimine is a direct result of the cytochrome P-450 dependent conversion. In in vitro incubations containing NAD(P)H, and in vivo, part of the benzoquinoneimine can be chemically reduced to give the para-hydroxyaniline. In vivo, this reduced form is sulphated or glucuronidated and excreted into urine. Results from in vivo urinary recovery studies with 2,3,4,5,6-penta- and 2,3,5,6-tetra-fluoroaniline demonstrate that in vivo this bioactivation of para-fluorinated anilines to reactive benzoquinoneimines as primary reaction products is of importance as well.
The biotransformation pathways proposed (see figure) might be of importance for bioactivation of other para-halogenated anilines, para-halogenated phenols and ortho-halogenated anilines or phenols.